Journal of the Lepidopterists' Society
46(3), 1992, 215–219

POTENTIAL FOR THE NON-INVASIVE STUDY OF INSECT HEART FUNCTION WITH A DOPPLER CRYSTAL SYSTEM

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ABSTRACT. This report describes a new technique for the non-invasive physiologic study of butterfly and moth circulatory systems. The technique employs a miniature Doppler crystal for *in vivo* assessment of circulatory function and provides information not obtained by previously reported methods.

Additional key words: blood flow, velocimeter, dorsal vessel, aorta, cardiac output.

The insect circulatory system has been studied extensively with reviews of structure and function (Davey 1964, Jones 1973, 1977, McCann 1970, Wigglesworth 1971). Although limited visual and photoelectric recordings of the insect heartbeat have been reported (Jones 1973, Tachibana & Nagashima 1957), most studies of *in vivo* function have required invasive electrical techniques (Miller 1973). Advances in miniature Doppler technology in the study of the human cardiovascular system can be applied to the study of insects. This report describes a new technique for the non-invasive evaluation of butterfly and moth circulatory systems.

METHODS

A 20 MHz Doppler catheter Model DC-201 (Millar Instruments, Inc., 6001-A Gulf Freeway, Houston, Texas 77223-0227) was used for transmitting and receiving acoustic signals. This catheter consists of a 20 MHz circular ceramic crystal attached to the tip of a USCI Rentrop Reperfusion Catheter, 135 cm length tapering to 1 mm diameter tip (Millar Instruments). This catheter is used for intravascular measurement of blood flow in the human coronary artery (Sibley et al. 1986). Two wires attached to the crystal traverse the lining of the catheter and are connected to a range-gated 20 MHz pulsed Doppler velocimeter (Millar Instruments) that detects the Doppler shift of the echoes from the blood cells. The velocimeter transmits pulses of 20 MHz ultrasound from the crystal at the catheter tip into the dorsal vessel. During the pause between pulses the crystal serves as a sensor and receives echoes from the blood cells. The distance between the crystal and the echo source may be varied from 1 to 10 mm by the range control on the velocimeter. The polarity of the phase shift (plus or minus 90 degrees) is determined by the direction of the motion either toward or away from the Doppler crystal. The pulsed Doppler velocimeter provides an audio signal with phasic output display. This phasic velocity signal from the pulsed Doppler velocimeter is recorded on a strip chart recorder

(Meda Sonics Model R 12 B, 340 Pioneer Way, Box 7268, Mountain View, California 94039). The strip chart recording displays not only the rate but the wave form of the Doppler shift frequency change at a specific distance from the transducer. Since the Doppler shift frequency is directly proportional to the velocity of the flow, the peak velocity can be measured at the peak of the wave form above the zero line (Hartley & Cole 1974). Audio recording was accomplished with a standard cassette tape recorder. The velocimeter was calibrated following the steps of the manufacturer (Millar Instruments).

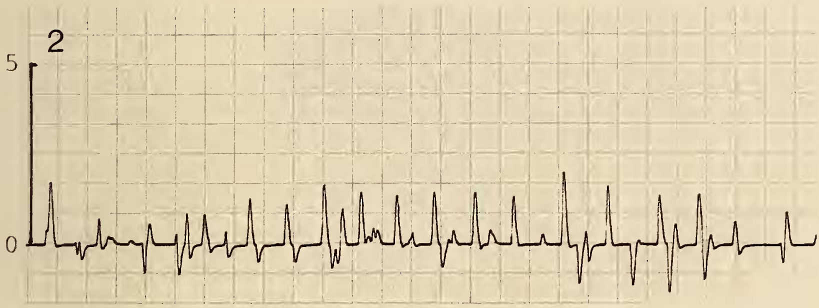
The butterfly or moth was placed on a standard mounting board where it was carefully positioned with wings spread and held down by paper strips. The body rested in the open midline groove but was not penetrated by pins. The tip of the Doppler catheter was then held over the dorsal midline upper abdomen or thorax lightly touching the insect. Conduction of the Doppler signal was enhanced by the use of a small amount of ultrasound transmission gel (Aquasonic 100—Parker Laboratories, Inc., Orange, New Jersey 07050) between the tip of the catheter and the body of the insect. The range control on the Doppler velocimeter was adjusted from 1 to 10 mm for sampling. Audio volume (loud, high signal to noise ratio) was used to guide the optimal sampling depth and angle of the catheter tip over the dorsal vessel with respect to the body of the insect.

RESULTS

Audible signals were recorded from the following species: *Epargyreus clarus* Cramer (Hesperiidae), *Limenitis arthemis* (Drury) (Nymphalidae), *Danaus plexippus* Linnaeus (Nymphalidae) over the thorax (aorta and/or pulsatile organ); and the moth *Catocala judith* Strecker (Noctuidae) over the upper abdomen (heart). At room temperature (22°C) heart rhythm was irregular in all species. The rate was variable at rest from as low as 20 to 30 per minute in *C. judith* (Fig. 1), to over 210 per minute in *E. clarus* (Fig. 2). The maximum peak velocity recorded was 2.0 cm/sec in *E. clarus*, 1.3 cm/sec in *L. arthemis*, and 1.5 cm/sec in *C. judith*. Because heart rate is variable, variations in peak velocity are expected. This variation was apparent at rapid rates (shortened diastole may impair adequate filling), but also occurred even at slower rates when filling should not be impaired. Reverse flow was noted by the negative deflection (below baseline) of the phasic wave form, generally following the positive flow wave (Fig. 2).

DISCUSSION

This report describes a new technique for the study of the butterfly and moth circulatory system. The technique potentially could be ap-



FIGS. 1 and 2. **1**, Doppler recording of *C. judith* heart activity. The maximum flow velocity for each heartbeat is the peak of the vertical distance (cm/sec) from the zero line. The paper speed is 25 mm/sec on the horizontal scale. The peak flow velocity on this recording is 1.2 m/sec; **2**, Doppler recording of *E. clarus* heart activity. The rate is rapid and the rhythm is irregular with variable peak velocities (cm/sec on the vertical scale). The deflections below the zero baseline indicate reverse flow. Paper speed on the horizontal scale is 25 mm/sec.

plied to other insects whose dorsal vessel is of sufficient size (minimal diameter unknown) to be within the resolution of the Doppler crystal frequency. The non-invasive feature of this technique minimizes trauma to the insect. Thus, multiple observations over time in the same subject may be performed. This technique avoids the artifactual changes that may be introduced with invasive *in vivo* techniques. Furthermore, the Doppler technique provides *in vivo* flow information not obtained by any other previously described method of study of the insect circulatory system. Using this technique, heart rate and flow parameters in the same subject could be obtained under varying conditions, or the rate and flow variables in different subjects could be compared under similar conditions. In addition, the flow per unit time can be calculated as the sum of the areas under the curves per unit of time (Cole & Hartley 1977, Perez 1987). Thus, if the radius of the vessel is known at the sample location, cardiac output (ml/min) can be calculated (Haites et al. 1985). Calculation of the forward cardiac output would require subtraction of the reverse flow per unit time.

Reviews of electrical and optical methods for recording the insect heartbeat are reported elsewhere (Jones 1977, Miller 1973). The variable heart rates of the Lepidoptera in this study are similar to those previously reported, but audio recordings have not been described.

Earlier studies of dorsal vessel muscle contraction reviewed elsewhere (Beard 1953, Miller 1985) have involved visual and invasive mechanical devices to study the peristaltic wave contraction. There are no previous studies of blood flow velocity in the dorsal vessel. This technique provides such information non-invasively.

Heartbeat reversal in which peristaltic waves of contraction are directed from front to back (reverse or retrograde peristalsis) are well described in insects since the first visual observation by Malpighia in 1669 (Gerould 1929, Davis 1961). This phenomenon has been attributed to changes in automaticity in cardiac pacemakers anteriorly and posteriorly, spontaneously or secondary to various stimuli (Davis 1961). Characteristically, anterograde (traveling anteriorly) peristaltic contractions occur for a period of beats then reverse or retrograde peristalsis occurs. This heartbeat reversal is to be distinguished from the reverse or backward blood flow pattern seen in the present study, usually immediately following the forward flow. This brief reverse flow is likely secondary to the characteristics of the open-end circulatory system. The hemolymph of insects is aspirated into the heart during diastole under a negative pressure (Wigglesworth 1971). Since the dorsal vessel is open-ended and without intraluminal valve structures to prevent back flow, some reverse flow is expected. Elastic recoil of the vessel wall and supporting structures may accentuate this effect (increased vascular compliance). However, this explanation cannot be proved without simultaneous Doppler flow pattern and electrical or visual observation of the peristaltic wave form.

The disadvantages of this technique include the immobilization of the insect, thus limiting conditions of the physiologic assessment. In addition, electrical artifact on the chart recorder at times limited the ability to record on paper the Doppler signal though it was easily audible. The etiology of the artifact is uncertain but likely is secondary to limitations of the strip chart recorder. A strip chart recorder with bioelectric amplifiers may reduce or eliminate this problem. Noise artifact may be produced by movement of the catheter tip on the insect body but this was not a significant problem. Occasional wing muscle contraction introduced noise artifact, most prominent in *E. clarus,* but this was usually brief and did not interfere with recordings. Gut movement did not produce noticeable noise artifact. Auditory artifact from electrical interference and its elimination from the velocimeter is detailed by the manufacturer (Millar Instruments). There was some difficulty in holding and positioning the catheter tip, especially for prolonged measurements. Measured flow rates may be underestimated by this technique with improper angulation between the transducer crystal and the vessel flow (Perez 1987).

Refinements and improvements in instrumentation specifically directed toward the study of the insect circulatory system should reduce these drawbacks. This technique offers an important new method for the non-invasive *in vivo* physiologic assessment of the butterfly and moth circulatory systems, and potentially other insects.

ACKNOWLEDGMENTS

I wish to thank Thomas Emmel for his review of the manuscript. I thank Larry Dean, David Sibley, and Mike Carter for their assistance in obtaining the equipment. Special thanks to Lyn Quillin for her assistance in preparation of the manuscript.

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Received for publication 18 November 1991; revised and accepted 10 May 1992.